



Establishment of a chronic left ventricular aneurysm model in rabbit

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Abstract

Objectives To establish a cost-effective and reproducible procedure for induction of chronic left ventricular aneurysm (LVA) in rabbits. **Methods** Acute myocardial infarction (AMI) was induced in 35 rabbits via concomitant ligation of the left anterior descending (LAD) coronary artery and the circumflex (Cx) branch at the middle portion. Development of AMI was confirmed by ST segment elevation and akinesis of the occluded area. Echocardiography, pathological evaluation, and agar intra-chamber casting were utilized to validate the formation of LVA four weeks after the surgery. Left ventricular end systolic pressure (LVESP) and diastolic pressure (LVEDP) were measured before, immediately after and four weeks after ligation. Dimensions of the ventricular chamber, thickness of the interventricular septum (IVS) and the left ventricular posterior wall (LVPW) left ventricular end diastolic volume (LVEDV), systolic volume (LVESV), and ejection fraction (EF) were recorded by echocardiogram. **Results** Thirty one (88.6%) rabbits survived myocardial infarction and 26 of them developed aneurysm (83.9%). The mean area of aneurysm was $33.4\% \pm 2.4\%$ of the left ventricle. LVEF markedly decreased after LVA formation, whereas LVEDV, LVESV and the thickness of IVS as well as the dimension of ventricular chamber from apex to mitral valve annulus significantly increased. LVESP immediately dropped after ligation and recovered to a small extent after LVA formation. LVEDP progressively increased after ligation till LVA formation. Areas in the LV that underwent fibrosis included the apex, anterior wall and lateral wall but not IVS. Agar intra-chamber cast showed that the bulging of LV wall was prominent in the area of aneurysm. **Conclusions** Ligation of LAD and Cx at the middle portion could induce development of LVA at a mean area ratio of $33.4\% \pm 2.4\%$ which involves the apex, anterior wall and lateral wall of the left ventricle.

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1 Introduction

Left ventricle aneurysm (LVA) is a common yet life-threatening complication that may occur after myocardial infarction. It is formed by a patch of weakened tissue in the ventricular free wall, which is inflated by the blood into a bubble. This area of the left ventricle is characterized by systolic dyskinesia or paradoxical bulging and thus is always coupled with reduction of the left ventricle ejection fraction. LVA could be fatal as it may cause ventricular failure or arrhythmia, or in some cases, thrombo-

sis of other parts of the body when the blood clots escapes from the aneurysm into the circulation.

Different surgical reconstruction techniques have been developed over last 60 years to treat LVA. In a study conducted by the international RESTORE Group who registered 1198 patients for the surgical treatment of LVA between 1998 and 2003 reported that the mean age of the patient was over 60.^[1] Thus, the need to improve the treatment and prognosis of LVA is especially urgent among the senior population. However, even as the current most effective treatment, surgical correction may cause secondary heart failure and the long-term survival rate is not optimal.^[1]

Improvement on the treatment relies on a profound understanding of the disease progression, thus requires establishment of highly accessible and reproducible animal models. Current available models are limited to large animals and may not be cost-effective, especially

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for long-term post-surgery investigation. The current study introduced a simple, reproducible and low-cost approach for inducing LVA in rabbits. Because left anterior descending (LAD) does not dominate the heart blood supply in rabbits, instead of using conventional occlusion of LAD alone to create myocardial infarction, circumflex (Cx) branch is also occluded at the same time at its mid region. We have demonstrated that the modified technique provides a higher rate of LVA development compared with the conventional approach.

2 Methods

2.1 Creation of acute myocardial infarction

Thirty five New Zealand White rabbits were subjected to myocardial infarction by coronary artery ligation and evaluated 4 weeks later to determine the presence of ventricular aneurysm. All animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 86-23, revised 1985). Animals were sedated with pentobarbital sodium (1mg/kg) without intubation. Under general anesthesia, a lower partial median sternotomy was performed. The pericardium was opened and the apex of heart was slightly elevated to facilitate exposure of LAD and Cx branch. Preconditioning was performed two times in order to reduce the risk of ventricular fibrillation after coronary artery ligation. LAD and middle portion of Cx were temporarily occluded by snare for 3 min using 6/0 polypropylene suture. After the 2 cycles of preconditioning, LAD and Cx branch were occluded permanently. Acute myocardial infarction was confirmed by ST segment elevation and immediate akinesis of the lateral wall as well as akinesis of the apex during systole. The chest was then closed when the hemodynamic status became stable. Penicillin was administered for three days post-surgery. Four weeks later, the animal was sacrificed for LVA investigation.

2.2 Validation of LVA formation

Three methods were used in combination to validate the LVA formation. Echocardiography was performed by an experienced investigator 4 weeks after surgery. Ventricular aneurysm was documented by the presence of bulging of the aneurysmal segment and dyskinetic wall motion (Figure 1 & 2). Animals only displaying hypokinetic myocardium of anterior and lateral wall but without developing LVA were excluded from further investigation.

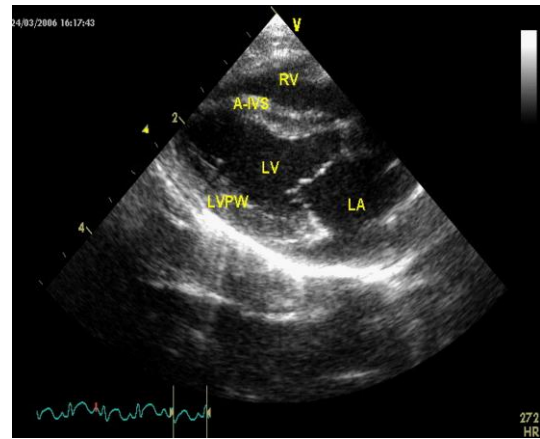


Figure 1. Normal heart from parasternal long-axis view.

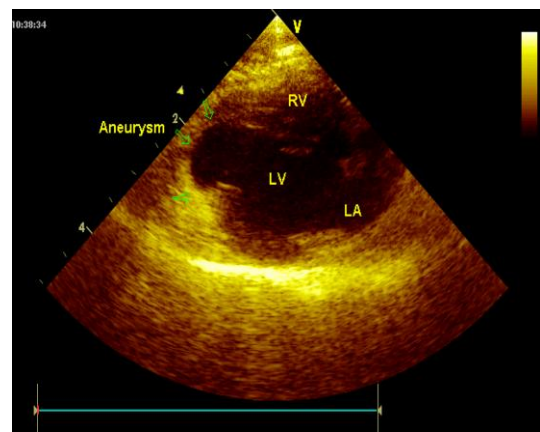


Figure 2. Four weeks after AMI, anterior and lateral LVA formation. AMI: acute myocardial infarction; LVA: left ventricular aneurysm.

After echocardiographic detection of LVA, the animal was sacrificed by injection of potassium chloride to arrest the heart at diastolic phase. Anatomic investigation showed myocardium of the apical and lateral wall was completely replaced by fibrous tissue. However, inter-ventricular septum (IVS) was not affected (Figure 3).

Agar intra-chamber cast was utilized to recapture the inner chamber spatial structure of left ventricle and the bulging shape of LVA. The animal was sacrificed as described above and the left atrium and mitral valve were excised from the heart. Liquid agar at 380°C was injected into left ventricle from the apex (the lowest part) to the level of mitral valve while the left ventricle was immersed in the water to prevent the weight-caused distention of the ventricle. Following solidification of agar, myocardium of left ventricle was cautiously removed, leaving the agar intact. The casted mold of agar showed the inner chamber shape of left ventricle.

2.3 Data collection

Echocardiography was performed prior to the procedure (baseline) and 4 weeks after the surgery. Dimensions of the ventricular chamber (D), thickness of IVS and left ventricular posterior wall (LVPW), left ventricular end diastolic volume (LVEDV) and systolic volume (LVESV), and ejection fraction (EF) were recorded (Table 1). Percentage of aneurysm to left ventricle was calculated.

Hemodynamic parameters, including left ventricular end systolic pressure (LVESP) and diastolic pressure (LVEDP), were directly measured by 20 gauges cannula via the apex at three time points: before (baseline), immediately after, and four weeks after coronary artery ligation (Table 2).

2.4 Statistical Analysis

All data are expressed as mean \pm SE. Differences in D, IVS, LVPW, LVEDV, LVESV, and EF were analyzed by SPSS software on two-way ANOVA and post-hoc test. $P < 0.05$ was considered to be statistically significant.

3 Results

Thirty one animals survived the coronary artery ligation resulting in a survival rate of 88.6%. Two animals died during operation because of inadvertent piercing into pleural cavity and subsequent acute pulmonary failure. Two animals died of ventricular fibrillation after the coronary ligation. Three animals developed heart failure as well as dyspnea and chest pleural effusion.

Among the survived, 26 out of 31 (83.9%) developed LVA, which was confirmed by pathologic investigation. Anatomic inspection showed myocardium of the apical and lateral wall was completely replaced by fibrous tissue. However, IVS was not affected (Figure 3). The shape of the casted agar revealed that the coniform apex was deformed after LVA formation (Figure 4-6). Echocardiography was also used to evaluate the development of LVA (Figure 1-2 and Table 1). The concordance of echocardiographic and anatomic docu-

mentation of LVA was 96.2%. Therefore, echocardiography is considered to be precise for LVA diagnosis. The average area of LVA was $33.4\% \pm 2.4\%$ (ranging from 30.3% to 36.7%) of the left ventricle. LV remodeling was manifested by significantly increased D2 and IVS thickness ($P < 0.01$). LVEDV and LVESV also increased as a result of apical and lateral myocardial infarction. Decreased LVEF demonstrated a compromised global systolic function. No statistical difference was found in D1 and LVPW thickness.



Figure 3. Anterior and lateral left ventricular wall was completely replaced by fibrous tissue. Interventricular septum was not affected.

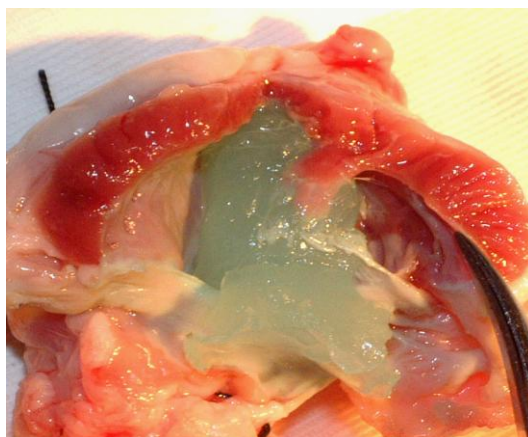


Figure 4. Intra-chamber agar casting of left ventricular.

Table 1. Structural parameter of LV prior to and after LVA formation.

	D1 (mm)	D2 (mm)	IVS (mm)	LVPW (mm)	LVEDV (mm ³)	LVESV (mm ³)	LVEF (%)
Baseline	22.4 \pm 0.7	13.6 \pm 1.5	0.25 \pm 0.02	0.28 \pm 0.01	2.77 \pm 0.07	0.89 \pm 0.13	67.9 \pm 4.7
LVA formation	16.4 \pm 2.3	26.6 \pm 1.7	0.34 \pm 0.04	0.31 \pm 0.03	3.93 \pm 0.58	2.29 \pm 0.28	43.8 \pm 5.5
<i>P</i> value	0.089	0.002	0.002	0.09	0.007	0.006	0.001

LV remodeling occurred after LVA formation resulting in increased LV dimension and volume and decreased EF. D1: LV dimension from parasternal long-axis view; D2: LV dimension from apex to mitral annulus on two chamber long-axis view; EF: ejection fraction; IVS: interventricular septum; LVEDV: left ventricular end diastolic volume; LVESV: left ventricular systolic volume; LVPW: left ventricular posterior wall.

Table 2. Hemodynamic parameters of LV prior to and after LVA formation.

	LVESDP (mmHg)	LVEDP (mmHg)
Baseline	50.596 ± 5.761	-9.165 ± 1.296
After ligation	38.739 ± 5.329*	-4.107 ± 1.115*
LVA formation	42.117 ± 5.036 [#]	-3.087 ± 1.476 [#]

Significantly changed hemodynamic parameters indicated compromised LV global systolic and diastolic function after coronary artery ligation and LVA formation. * $P < 0.05$ vs. Baseline; [#] $P < 0.05$ vs. After ligation. LV: left ventricular; LVA: left ventricular aneurysm; LVEDP: left ventricular end diastolic pressure; LVESP: left ventricular end systolic pressure.

**Figure 5. Normal spatial structure of coniform left ventricular.****Figure 6. Bulging of anterior and lateral wall of left ventricular after aneurysm formation.**

Hemodynamic parameters showed compromised LV function that was exacerbating as the disease progresses. Immediately after ligation, LVESP dramatically lowered to 38.739 ± 5.329 mmHg from a baseline of 50.596 ± 5.761 mmHg. Four weeks later, LVESP slightly increased to 42.117 ± 5.036 mmHg but did not recover to the baseline level. LVEDP progressively increased from the baseline level of -9.165 ± 1.296 mmHg to -4.107 ± 1.115 mmHg after AMI and to -3.087 ± 1.476 mmHg after LVA formation four weeks later (Table 2).

4 Discussion

Surgical treatment is the most effective approach to manage LVA. However, the long-term outcome is not optimal, with 5-year survival rate varying between 58% and 80%,^[2,3] and 10-year overall survival being about 34%.^[2] Cardiac causes are responsible for 57% of the late deaths.^[4] Many patients suffer from recurrent heart failure that starts months or years after surgery. Unfortunately, the mechanism of the late heart failure has not been elucidated. Therefore, establishing an animal model that imitates the clinical course of LVA is essential for advancing our understanding on the pathological change in the heart with LVA.

As compared to the LVA model induced in sheep and rats,^[5,6] establishing LVA model in rabbits offers several advantages. It has a higher success rate, better experimental consistency compared to rats, easier handling and lower cost to maintain when compared to sheep. However, the conventional procedure of LAD ligation in the rabbit heart, did not lead to immediate hypokinesis/akinesis of LV wall followed by infarction, which is usually observed in patients after acute occlusion of LAD. Thus, this conventional technique to establish AMI model was modified in the present study. We found that concomitant ligation of LAD and Cx branch at its middle portion resulted in immediate large area of akinesis of apical and lateral LV wall with the typical ST segment elevation which represents AMI. With this technique, 83.9% of animal developed LVA four weeks later.

Moreover, techniques were further optimized to reduce the mortality rate. One of the challenges of this technique is that direct ligation of LAD and Cx proximal to its middle point may cause malignant ventricular fibrillation and acute heart failure. To minimize the mortality of the animal, myocardial preconditioning was employed to reduce the ventricular arrhythmia.^[7,8] In the event that large area of akinesis occurred immediately after coronary occlusion, ligation suture was moved dis-

tally at once. Other procedures that are critical to the success of the surgery includes: (1) prior to sternotomy, xiphoid was transected to decrease bleeding; (2) inadvertent entrance into pleural cavity should be avoided as it may lead to acute pulmonary failure and animal death; and (3) prior to chest closure, pericardium approximation with lubrication placed around the heart may benefit in subsequent repeat operations.

Left ventriculography is the “gold standard” for diagnosis of LVA. Yet, this approach is not readily applicable on small animals. Another approach sensitive and specific for LVA diagnosis is echocardiography, which is commonly used on patients as a more accessible alternative. In this study, echocardiography, in combination with pathological anatomic investigation and intra-chamber agar casting, was used to confirm LVA formation. We found the concordance of echocardiography documentation compared with anatomic detection of LVA was 96.2%, indicating the sensitivity and specificity of echocardiography for LVA diagnosis.

In this study, LVA formed with a relatively consistent area ratio of $33.4\% \pm 2.4\%$. Anatomic evaluation revealed that apical and lateral wall were replaced by fibrous tissue but IVS was not affected. The mechanism is not clear. This is not consistent with the observation of patients whose IVS were usually involved in the fibrosis after LAD occlusion. However, this might be a benefit for off-pump reconstruction research of LVA since IVS is not affected.

Means to evaluate the spatial geometric structure of remodeling LV after AMI include echocardiogram, magnetic resonance imaging, and computed tomography. Intra-chamber agar casting was used in this study to directly show the inner chamber 3-D structure, providing the direct evidence of LV remodeling after LVA formation. The preliminary results demonstrated that the bulging of anterior lateral wall was accompanied by the chamber dilatation and distortion, which occurred

secondarily to the LVA formation. Hence, this casting approach may facilitate geometric reconstruction research of LVA which aims to restore the normal conal shape of LV.

In summary, concomitant ligation of LAD and circumflex branch is a simple, cost effective, and reproducible approach to induce chronic LVA in rabbit, and may also be potentially useful for off-pump reconstruction research of LVA in rabbit.

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